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(54) [Title of the Invention] METHOD FOR MANUFACTURING TEA
CATECHIN

(57) [Abstract]

[Object]

In order to manufacture tea catechins safely from food-hygienic point of view in an industrial scale, there is provided a method for manufacturing tea catechins of high purity at a low cost by removing saccharides, amino acids, and other impurities, and also caffeine and catechin oxidative polymerization products contained in teas, without conducting a liquid-liquid extraction process.

[Constitution]

The method includes an extraction process for obtaining a tea extract liquid from tea leaves; an adsorption process for adsorbing tea catechin components to a chromatography column packed with a gel-type synthetic adsorbent; a washing process for removing water-soluble impurities including at least

caffeine, other than tea catechins, by washing the chromatography column with water; and an elution process for eluting components remaining in the chromatography column with an eluant of aqueous solution containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[Claims for the Patent]

[Claim 1]

A method of manufacturing tea catechins comprising:

an extraction process for obtaining a tea extract liquid by extracting water-soluble components from tea leaves;

an adsorption process for adsorbing tea catechin components to a chromatography column packed with a gel-type synthetic adsorbent by injecting the tea extract liquid into the chromatography column;

a washing process for removing water-soluble impurities including at least caffeine, other than tea catechins, by washing the chromatography column with water; and

an elution process for eluting components remaining in the chromatography column with, as an eluant, an aqueous solution containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[Claim 2]

The method of manufacturing tea catechins according to claim 1, characterized by removing at least a catechin oxidative polymerization product in the washing process.

[Claim 3]

The method of manufacturing tea catechins according to claim 1, characterized in that the main component of the gel-type adsorbent is a vinyl polymer.

[Claim 4]

The method of manufacturing tea catechins according to claim 1, characterized in that the amount of the tea catechins is at

least 80 wt% of the solid contents in the liquid recovered from the chromatography column in the elution process.

[Claim 5]

The method of manufacturing tea catechins according to claim 1, characterized in that the amount of the tea catechins is at least 90 wt% of the solid contents in the liquid recovered from the chromatography column in the elution process.

[Claim 6]

The method of manufacturing tea catechins according to claim 1, characterized by removing at least 80% of caffeine and 90% of catechin oxidative polymerization products by washing in the washing process.

[Claim 7]

The method of manufacturing tea catechins according to claim 1, characterized in that the volume ratio of the loaded tea extract liquid to the adsorbent is 0.5 to 6 when the concentration of the tea extract liquid is converted into 15 Brix%.

[Claim 8]

The method of manufacturing tea catechins according to claim 1, characterized in that the volume ratio of the water in the washing process to the adsorbent is 2 to 6.

[Claim 9]

The method of manufacturing tea catechins according to claim 1, characterized in that a volume ratio of the eluant to the adsorbent is 2 to 6.

[Claim 10]

The method of manufacturing tea catechins according to claim 1, characterized in that the adsorbent is a hydrophilic vinyl polymer, the volume ratio of the loaded tea extract liquid to the adsorbent is 1 when the tea extract liquid concentration is converted into 15 Brix%, the adsorbent is washed with water in a volume ratio of 4 with respect to the adsorbent, and 55 vol% ethanol aqueous solution in a volume ratio of 3 with respect to the adsorbent passing through the adsorbent.

[Claim 11]

The method of manufacturing tea catechins according to claim 1, characterized in that the tea catechins are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, (+)-catechin, (+)-gallocatechin, and (+)-gallocatechin gallate.

[Claim 12]

A method of manufacturing tea catechins comprising:

an extraction process for obtaining a tea extract liquid by extracting water-soluble components from tea leaves;

an adsorption process for adsorbing tea catechin components to a chromatography column packed with a synthetic adsorbent by injecting the tea extract liquid into the chromatography column;

a washing process for removing water-soluble impurities including at least caffeine, other than tea catechins, by washing the chromatography column with water and hot water sequentially; and

an elution process for eluting components remaining in the chromatography column with an eluant of an aqueous solution

containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[Claim 13]

The method of manufacturing tea catechins according to claim 12, characterized in that the main component of the synthetic adsorbent is styrene divinylbenzene or methacrylic acid ester.

[Claim 14]

The method of manufacturing tea catechins according to claim 12, characterized in that the temperature range of the hot water is 60 to 90°C.

[Claim 15]

The method of manufacturing tea catechins according to claim 12, characterized in that the concentration of the aqueous solution of one of methanol, ethanol, and acetone solvents or a mixture thereof is 50 to 100%.

[Detailed Description of the Invention]

[0001]

[Industrial Application Field]

The present invention relates to a method of manufacturing tea catechins and, more specifically relates to a method of manufacturing tea catechins of high purity at a low cost by using tea leaves as raw materials and removing caffeine and the like.

[0002]

[Conventional Art]

Tea is one of favorite drinks that are widely drunk all over the world and is a woody evergreen plant botanically belonging to the genus *Camellia* in the family *Theaceae*. Teas are roughly

classified into green tea (unfermented tea), oolong tea (semi-fermented tea), and black tea (fermented tea) according to the method of manufacturing drinkable tea, but, basically, these products can be obtained from the same tea tree.

[0003]

Tea catechins contained in tea leaves are polyphenol compounds and are (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, (+)-catechin, (+)-gallocatechin, and (+)-gallocatechin gallate. In a general sense, the tea catechins include oligomers (hereinafter referred to as "catechin oxidative polymerization products"), such as theaflavins in which the above catechins are condensed as structural components. Tea leaves contain 9 to 30 wt% tea catechins.

[0004]

Among these catechins, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate are four main catechins in tea leaves (hereinafter referred to as "tea catechins"). Recently, research on these catechins has confirmed that tea catechins have chemical and pharmacological activities such as anti-oxidative, anti-bacteria, deodorization, reduction of blood cholesterol, reduction of blood pressure, and anti-tumor activity, and it is expected to develop application of the catechins to food materials, functional foods, and so on.

[0005]

Furthermore, the above-mentioned tea catechins as the object of the present invention may include (+)-catechin, (+)-gallocatechin, and (+)-gallocatechin gallate, in addition to the

four main catechins in tea leaves: (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate.

[0006]

Regarding the catechin oxidative polymerization products, examples thereof include proanthocyanidins, assamicains, oolonghomobisflavans, hydrolyzed tannin, theaflavins, theaflagallins, and theasinensins.

[0007]

In order to manufacture such tea catechins so as to have high purity, impurities coexistence with the above-mentioned catechins in tea leaves must be isolated. The impurities are caffeine, saccharides, amino acids, protein, organic acids, lipids, pigments, catechin oxidative polymerization products, and so on.

[0008]

Among these components, the catechin oxidative polymerization products are classified into tea catechins in a general sense, but do not have activities such as anti-oxidation. In addition, they are brown and may cause a problem of color development of end products containing such catechin oxidative polymerization products.

[0009]

Therefore, it is desirable that the catechin oxidative polymerization products be removed not to be contained in the tea catechins as an object that is manufactured in the present invention.

[0010]

Other impurities such as saccharides and amino acids become nutrition sources of general organisms and, therefore, are desirably removed for sufficiently exhibiting various activities of tea catechins.

[0011]

Conventionally, tea catechins are manufactured by a method using liquid-liquid distribution extraction.

[0012]

For example, Yoshida (Chagyo Kenkyu Hokoku (Tea Research Journal), 1959 April, No. 13, p. 4) reports a method by extracting green tea with water, removing caffeine with chloroform, and conducting extraction with ethyl acetate.

[0013]

Furthermore, Japanese Patent Publication No. 1-44234 describes that natural antioxidative agent can be manufactured in an industrial scale by a similar method to the above.

[0014]

Furthermore, Japanese Patent Laid-Open No. 64-9922 describes that target tea catechins can be obtained by removing caffeine from a tea extract liquid with hexane and chloroform and extracting the resultant with ethyl acetate.

[0015]

In these methods, a tea extract as the starting material contains many impurities, and extraction efficiency is low because of the extraction distribution coefficient. Therefore, it is thought that extraction is necessarily repeated to obtain target tea catechins at high purity.

[0016]

In addition, since chloroform, which is a chlorine-based organic solvent, is used for removing caffeine, problems concerning safety are anticipated when applied to foods and the like.

[0017]

Furthermore, Japanese Patent Laid-Open No. 3-14572 describes a method manufacturing tea catechins including a catechin polymer or complex. In this method, a substance obtained by extraction of a plant, such as tea, with a lower alcohol aqueous solution is used as a raw material, and a multi-stage batch process using gel beads, which can be performed under high loading conditions, is employed.

[0018]

The product manufactured by this method contains a catechin polymer or complex, and the gel beads are anticipated to be worn by stirring or shaking the inside of a reaction tank during the manufacturing.

[0019]

On the other hand, the present inventors have developed chromatography column adsorption/separation technique for manufacturing tea catechins of high purity. In this method, a tea extract obtained by extracting tea leaves with hot water is used as a raw material. Tea catechins are selectively adsorbed on an adsorbent packed in a chromatography column, and tea catechins are eluted with a hydrophilic organic solvent and recovered (Japanese Patent Laid-Open No. 2-311474).

[0020]

According to this method, impurities are previously removed by injecting a tea extract (water-soluble components) to a chromatography column packed with an adsorbent and sequentially eluting with water and a hydrophilic organic solvent while changing the concentrations. Tea catechins as the target component can be recovered at high purity and a high recovery rate by eluting the components remaining in the chromatography column with an aqueous solution containing about 10 to 65 vol% of one of methanol, ethanol, and acetone or a mixture thereof.

[0021]

In particular, among the impurities, caffeine is reported to cause disorders by excessive intake thereof and influence on newborns, though it does not cause problems by usual intake. Therefore, it is required depending on types of foods to eliminate caffeine when tea catechins are manufactured.

[0022]

The caffeine content in tea leaves is about 2 to 5 wt%, though it varies depending on the type of tea. In a tea extract liquid obtained by extracting tea leaves with hot water, the caffeine content is about 4 to 10 wt%, when converted into solid content.

[0023]

If tea catechins are manufactured using this tea extract liquid without removing caffeine, the content is further increased to about 10 to 25 wt%.

[0024]

The present inventors have investigated optimum conditions for removing caffeine in the technique developed in the

aforementioned invention of Japanese Patent Laid-Open No. 2-311474 with the view of applying caffeine-removed tea catechins to foods used for infants, pregnant women, persons with liver dysfunction, and others who are thought that caffeine is unpreferable and to foods for livestock or pet animals that are sensitive to caffeine.

[0025]

As a result of this investigation, it has been confirmed that caffeine-removed tea catechins can be manufactured, without using chloroform, by optimizing the concentration and the amount of a hydrophilic organic solvent used in the washing-out of impurities for the respective adsorbents used.

[0026]

However, the removal of caffeine requires a large amount of aqueous solution containing a solvent at a low concentration of about 5 to 15 wt%. This causes another problem of increasing the cost for collecting the solvent.

[0027]

Furthermore, subsequent research revealed that the thus manufactured products contain catechin oxidative polymerization products that are brown and do not have activities such as anti-oxidation.

[0028]

Accordingly, first, in an analysis level for understanding behavior of catechin oxidative polymerization products from the time they are a raw material till the time they are an end product, new separation and analysis technique was developed. Then, a method for removing catechin oxidative polymerization

products in an industrial level was further investigated. As a result, a method for manufacturing tea catechins in which catechin oxidative polymerization products can be removed at high efficiency has been developed (Japanese Patent Laid-Open No. 4-182479). The method is accomplished by organically combining a separation process of chromatography separation with a liquid-liquid extraction process.

[0029]

According to this method, for example, since at least impurities other than catechin oxidative polymerization products are previously removed in the separation process using a chromatography column, and, therefore, the catechin oxidative polymerization products can be removed using a small amount of an extraction solvent in the subsequent liquid-liquid extraction process. Thus, tea catechins of high purity can be manufactured.

[0030]

Tea catechins can be manufactured efficiently at high purity and a high recovery rate by thus combining a chromatography separation process and a liquid-liquid extraction process, compared to a case that these processes are individually conducted. However, the entire processes in the manufacturing are necessarily complicated. This complication in the manufacturing processes is another problem on a manufacturing line.

[0031]

[Problems to be Solved by the Invention]

The present invention has been made from the viewpoint of the above problems, and an object of the present invention is to

provide a method of manufacturing tea catechins by using tea as a raw material in an industrial scale safely from food-hygienic point of view, in particular, manufacturing tea catechins of high purity at a low cost by removing not only caffeine and impurities such as saccharides and amino acids but also catechin oxidative polymerization products contained in teas, without conducting a liquid-liquid extraction process.

[0032]

[Means for Solving the Problems]

According to the present invention, caffeine, catechin oxidative polymerization products, and the like contained in tea leaves can be removed efficiently and inexpensively to manufacture tea catechins of high purity. Various investigations have been conducted from the viewpoint of the current status that the solvent generated in a process for removing water-soluble impurities must be collected. As a result, a method for washing the water-soluble impurities without using a solvent has been found. The present invention has been accomplished based on this finding.

[0033]

That is, a first aspect of the invention has been accomplished from the viewpoint of selection of a synthetic adsorbent to be packed in a chromatography column and washing of the chromatography column with water, without using an organic solvent. That is, the first aspect of the invention provides a method of manufacturing tea catechins, including an extraction process for obtaining a tea extract liquid by extracting water-soluble components from tea leaves; an adsorption process for

adsorbing tea catechin components to a chromatography column packed with a gel-type synthetic adsorbent by injecting the tea extract liquid into the chromatography column; a washing process for removing water-soluble impurities including at least caffeine, other than tea catechins, by washing the chromatography column with water; and an elution process for eluting components remaining in the chromatography column with an eluant of aqueous solution containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[0034]

In the aforementioned washing process, at least catechin oxidative polymerization products can be removed.

[0035]

The aforementioned gel adsorbent can have the main component of a vinyl polymer.

[0036]

In the aforementioned elution process, the amount of the obtained tea catechins can be at least 80 wt% of the solid contents in the liquid collected from the chromatography column.

[0037]

In the aforementioned elution process, the amount of the obtained tea catechins can be at least 90 wt% of the solid contents in the liquid collected from the chromatography column.

[0038]

In the aforementioned washing process, at least 80% of caffeine and 90% of catechin oxidative polymerization products can be removed by washing with water.

[0039]

The amount of the aforementioned tea extract liquid loaded can be 0.5 to 6 in a volume ratio to the adsorbent, when the tea extract liquid concentration is converted into 15 Brix%.

[0040]

The volume ratio of the water in the aforementioned washing process to the adsorbent can be 2 to 6.

[0041]

The volume ratio of the aforementioned eluant to the adsorbent can be 2 to 6.

[0042]

Furthermore, a hydrophilic vinyl polymer can be used as the aforementioned adsorbent. The amount of the tea extract liquid loaded may be 1 in a volume ratio to the adsorbent, when the tea extract liquid concentration is converted into 15 Brix%. The adsorbent may be washed with water in a volume ratio of 4 with respect to the adsorbent, and 55 vol% ethanol aqueous solution in a volume ratio of 3 with respect to the adsorbent may be applied.

[0043]

The aforementioned tea catechins may be (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, (+)-catechin, (+)-gallocatechin, and (+)-gallocatechin gallate.

[0044]

Next, a second aspect of the invention has been accomplished from the viewpoint of washing of the chromatography column to which caffeine and tea catechins are adsorbed thereon with hot water. The second aspect of the invention provides a method of

manufacturing tea catechins, including an extraction process for obtaining a tea extract liquid by extracting water-soluble components from tea leaves; an adsorption process for adsorbing tea catechin components to a chromatography column packed with a synthetic adsorbent by injecting the tea extract liquid into the chromatography column; a washing process for removing water-soluble impurities, other than tea catechins, including at least caffeine by washing the chromatography column with water and hot water sequentially; and an elution process for eluting components remaining in the chromatography column with an eluant of an aqueous solution containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[0045]

The main component of the aforementioned synthetic adsorbent can be styrene divinylbenzene or methacrylic acid ester.

[0046]

The temperature range of the aforementioned hot water can be 60 to 90°C.

[0047]

The concentration of the aforementioned aqueous solution of one of methanol, ethanol, and acetone solvents or a mixture thereof can be 50 to 100%.

[0048]

The present invention will now be more specifically described. The invention provides a method of manufacturing tea catechins, briefly, including the processes of applying a tea extract liquid that is extracted from tea leaves with, for example, hot water and contains water-soluble components to a

chromatography column packed with an adsorbent; washing impurities including caffeine and catechin oxidative polymerization products with water; and collecting the target tea catechins remaining in the chromatography column by desorbing the tea catechins with an aqueous solution containing 50 to 100 % of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[0049]

Figure 1 schematically shows processes of the method of manufacturing tea catechins according to the first aspect of the invention.

[0050]

The aforementioned target tea catechins are a mixture of (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate, which are relatively highly contained and main components for various effects, but may further contain (+)-catechin, (+)-gallocatechin, and (+)-gallocatechin gallate, which are minor components.

[0051]

The aforementioned adsorbent is desirably a gel filtration adsorbent having the main component of a hydrophilic vinyl polymer.

[0052]

More specifically, teas in various forms such as raw tea, unfermented tea, semi-fermented tea, or fermented tea can be used as a raw material of the present invention. Tea is extracted, for example, with hot water of 80°C or higher for

about 5 to 15 minutes to give about 2 to 4 Brix% tea extract liquid containing water-soluble components.

[0053]

This tea extract liquid may be used after concentration according to necessity or may be used directly without concentration.

[0054]

In a case of concentrating the tea extract liquid, the liquid is concentrated to about 15 Brix% at the maximum in order to avoid the generation (cream down) of precipitate by a reaction of tea catechins and caffeine.

[0055]

That is, the concentration of the tea extract liquid used in the present invention is about 2 to 15 Brix%.

[0056]

In addition, a tea extract liquid may be prepared by drying the concentrated tea extract liquid into a solid powder and then dissolving the solid powder in water again.

[0057]

If the concentration of a tea extract liquid is high, namely, if the concentration of tea catechins is high, the adsorption equilibrium amount is relatively high and the processing amount per unit of an adsorbent in a chromatography column increases. However, since the viscosity of the tea extract liquid also increases, the pressure loss in the chromatography column increases.

[0058]

On the other hand, in a tea extract liquid with a low concentration not undergoing a concentration process, the adsorption equilibrium amount is small, and the processing amount per unit of an adsorbent in a chromatography column decreases. However, the pressure loss in the chromatography column is small, and it is therefore preferable for chromatography column design.

[0059]

Therefore, it is suitably determined whether the concentration of a tea extract liquid is necessary or not, by taking raw-material-supplying conditions and restriction in device design into consideration.

[0060]

The thus prepared tea extraction liquid is applied to a chromatography column packed with an adsorbent.

[0061]

The adsorbent packed in a chromatography column can be a gel-type synthetic adsorbent such as gel filtration adsorbent of which main component is a vinyl polymer such as a hydrophilic vinyl polymer.

[0062]

For example, Toyopearl HW40EC (manufactured by Tosoh Corp.) or Asahipak GS-520 (manufactured by Showa Denko K.K.) can be used.

[0063]

In addition, adsorbents of which main components are styrene divinylbenzene or a methacrylic acid ester are difficult to separate tea catechins from caffeine, and in particular, from

catechin oxidative polymerization products under the same conditions as those when an adsorbent with the main component of a hydrophilic vinyl polymer is used.

[0064]

Furthermore, tea catechins can be separated from caffeine and catechin oxidative polymerization products by using an adsorbent with the main component of a dextran derivative. However, since such adsorbents have a small particle diameter, as in Sephadex LH-20 (manufactured by Pharmacia), it is anticipated to cause problems such as a large loss in the pressure when a liquid passes through the column is large and consolidation of the packed bed.

[0065]

The loading amount of the tea extract liquid on a chromatography column is desirably determined by previously measuring the amount of tea catechins at the breakthrough by injecting each concentration of a tea extract liquid and using this measurement value as a benchmark.

[0066]

The loading amount is determined so as to be 20 to 95% of the loading amount of the breakthrough point, in consideration of a slight shift in the absorbance band of the tea catechins in the sequential washing process with water.

[0067]

When the loading amount is lower than 20%, the production efficiency per adsorbent amount is decreased. When the loading amount is higher than 95%, a loss of about 15% or more in tea

catechins is caused depending on application conditions such as column design and a flow rate.

[0068]

Furthermore, when a tea extract liquid is loaded at an amount exceeding the breakthrough point, the tea extract liquid is not adsorbed to the adsorbent and flows out from the column to significantly decrease the recovery rate of the tea catechins.

[0069]

The present inventors have conducted intensive studies on loading amounts of tea extract liquids in consideration of separation characteristics of target tea catechins from, in particular, caffeine and catechin oxidative polymerization products and, as a result, have found that the amount of a tea extract liquid loaded should be in a volume ratio of 0.5 to 6 to a synthetic adsorbent when the tea extract liquid concentration is converted into 15 Brix%.

[0070]

The loading amount of such a tea extract liquid is desirably 50 to 360 mg of solids/mL resin when it is expressed as weight of the water-soluble solid in a tea extract liquid per unit volume of the adsorbent.

[0071]

When the loading amount of a tea extract liquid is higher than 360 mg of solids/mL-Resin, the absorbance band of tea catechins in the adsorbent layer becomes too long, and therefore tea catechins are rendered breakthrough together with caffeine in the sequential washing process using water to decrease the recovery rate of tea catechins. In addition, in a tea extract

liquid of a high concentration, a pressure loss in the chromatography column is increased by influences of impurities.

[0072]

When the loading amount of a tea extract liquid is less than 50 mg of solids/mL-Resin, tea catechins can be separated from caffeine. However, the manufacturing amount is merely decreased, and, therefore, it is uneconomic.

[0073]

Thus, impurities that are unadsorbable to an adsorbent, such as saccharides and amino acids, are discharged from the column outlet without being adsorbed to the adsorbent by applying a tea extract liquid to the column.

[0074]

In addition, when certain amounts of caffeine and catechin oxidative polymerization products are weakly adsorbed to the adsorbent, breakthrough begins and the caffeine and catechin oxidative polymerization products are eluted out from the column outlet.

[0075]

The target tea catechins can be held on the adsorbent without causing breakthrough when the loading amount does not exceed the aforementioned level.

[0076]

At the point when a predetermined amount of a tea extract liquid has been applied to a chromatography column, tea catechins and parts of caffeine and catechin oxidative polymerization products are adsorbed on the adsorbent in the

chromatography column, and impurities remain in gaps of the adsorbent particles.

[0077]

Then, the chromatography column is washed with water. This washing process discharges the unadsorbable impurities such as saccharides and amino acids remaining in the gaps of the adsorbent when the injection of the tea extract liquid has been finished.

[0078]

In addition, caffeine and catechin oxidative polymerization products that are weakly adsorbed to the adsorbent are desorbed from the adsorbent and are eluted out from the column with a change in the concentration balance between the mobile phase and the stationary phase.

[0079]

The amount of water used in this washing process is preferably 2 to 6 in a volume ratio to the synthetic adsorbent.

[0080]

When the volume ratio is less than 2, caffeine and catechin oxidative polymerization products are insufficiently removed. When the volume ratio is higher than 6, the recovery rate of tea catechins is decreased due to partial breakthrough of the tea catechins.

[0081]

In this washing process using water, tea catechins are not lost from the chromatography column. The whole quantity of tea catechins can be collected in the subsequent eluent procedure (elution process).

[0082]

That is, tea catechins adsorbed and remaining in the column are desorbed using a hydrophilic organic solvent, for example, an eluant of aqueous solution containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof, and are eluted out from the column.

[0083]

The hydrophilic solvent used is desirably a single solvent of ethanol from the viewpoint of addition to a food and a solvent-collecting process.

[0084]

A solvent concentration of 50 vol% or less is unpreferable, because the elution rate of tea catechins is reduced, and the recovery rate of tea catechins is also decreased.

[0085]

The amount of the eluant used in this elution process is preferably 2 to 6 in a volume ratio to the synthetic adsorbent.

[0086]

When the volume ratio is less than 2, the recovery rate of tea catechins becomes insufficient. When the volume ratio is higher than 6, the amount of the collected tea catechins becomes a small quantity due to the eluant.

[0087]

The polarity of the mobile phase is shifted by these solvents, and tea catechins are easily desorbed from the adsorbent and are discharged from the chromatography column outlet.

[0088]

At a solvent concentration of lower than 60 vol%, danger such as firing is reduced, and therefore it is advantageous in manufacturing control. When a solvent concentration is lower than 50 vol%, the elution rate becomes slow.

[0089]

Therefore, the solvent concentration design may be properly determined in consideration of restriction in device design.

[0090]

Furthermore, when the solvent amount is less than 1.5 times the adsorbent volume in a solvent standard, tea catechins cannot be sufficiently eluted out.

[0091]

Lastly, the chromatography column is washed with water to collect the solvent remaining in the column.

[0092]

The thus collected tea catechins are subjected to concentration and drying processes in a usual manner and are made into products in a concentrated liquid or dried powder form.

[0093]

Next, the second aspect of the invention will be described. The invention is a method of manufacturing tea catechins, briefly, by injecting a tea extract liquid extracted with hot water into a chromatography column packed with a synthetic adsorbent, washing the chromatography column with only water first and then removing caffeine with hot water of 60 to 95°C, and eluting out the components remaining in the chromatography column with an aqueous solution containing 50 to 100 vol% of one of methanol, ethanol, and acetone solvents or a mixture thereof.

[0094]

Figure 2 schematically shows processes of the method of manufacturing tea catechins according to the second aspect of the invention.

[0095]

After the application of a tea extract liquid to a chromatography column, impurities that are solidified by hot water are first removed by washing the column with water before the washing with hot water.

[0096]

When the temperature of this washing hot water is 60°C or lower, the removal of caffeine is insufficient. When the temperature is 95°C or higher, the liquid ununiformly flows because of foams generated in the chromatography column.

[0097]

First, the washing with water separates impurities other than caffeine from the water-soluble components of tea leaves by the synthetic adsorbent packed in the chromatography column.

[0098]

These impurities have a property that they are hardly adsorbed to the adsorbent. The impurities remaining in gaps in the chromatography column when a tea extract is applied to the column can be discharged to the outside of the column by washing away with water.

[0099]

Since caffeine is adsorbed to the adsorbent with relatively high adsorption power compared to those of other impurities, the elution rate with water is slow. However, the elution rate can

be increased by using hot water, and caffeine can be discharged to the outside of the chromatography column.

[0100]

On the other hand, tea catechins have high adsorption power to the adsorbent and therefore are not eluted with water or hot water and can be desorbed from the adsorbent and collected by using an aqueous solution of a hydrophilic organic solvent having a high solvent polarity, as in the first aspect of the invention.

[0101]

[Operation]

In the method of manufacturing tea catechins according to the present invention, tea catechins of high purity can be inexpensively manufactured by efficiently removing caffeine and catechin oxidative polymerization products in the first aspect of the invention or efficiently removing caffeine in the second aspect of the invention.

[0102]

Since water or hot water is used for separating caffeine and catechin oxidative polymerization products, collection of organic solvents used for eluting caffeine and catechin oxidative polymerization products is unnecessary. Therefore, the manufacturing facilities can be simplified.

[0103]

[Examples]

The present invention will now be described with reference to Examples. Before the description, a calculation method for quantitative determination of tea catechins will be described.

[0104]

In the separation process using a chromatography column, the following values: (1) each component amount (mass) in a tea extract liquid injected into the column, (2) each component amount in a recovered liquid obtained in an elution process, (3) each component amount ratio of these liquids, and (4) solid weights obtained by removing solvents of the tea extract liquid and the recovered liquid, are defined as follows:

[0105]

(1) Each component amount in a tea extract liquid

Ct(in): catechin

Co(in): catechin oxidative polymerization products

Cf(in): caffeine

Ci(in): impurities

(2) Each component amount in a recovered liquid in an elution process

Ct(out): tea catechins

Co(out): catechin oxidative polymerization products

Cf(out): caffeine

Ci(out): impurities

(3) Each component amount ratio = (each component amount in a recovered liquid in an elution process)/(each component amount in a tea extract liquid)

η_t : Ct(out)/Ct(in)

η_o : Co(out)/Co(in)

η_f : Cf(out)/Cf(in)

η_i : Ci(out)/Ci(in)

(4) Solid weight in the tea extract liquid: W(in)

$W(in) = Ct(in) + Co(in) + Cf(in) + Ci(in) \dots$ Equation ①
Solid weight in the recovered liquid in the elution process:

$W(out)$

$W(out) = Ct(out) + Co(out) + Cf(out) + Ci(out) \dots$ Equation
②

[0106]

The purity of tea catechins contained in the solid contents in the eluate obtained by the chromatography separation process can be expressed by the following Equations ③ or ④:

$\eta_t \cdot Ct(in) / (\eta_t \cdot Ct(in) + \eta_o \cdot Co(in) + \eta_f \cdot Cf(in) + \eta_i \cdot Ci(in)) \times$
100 ... Equation ③

$Ct(out) / W(out) \times 100 \dots$ Equation ④

[0107]

Tea catechins and caffeine can be quantitatively determined by high-performance liquid chromatography (hereinafter referred to as "HPLC") by using standard samples, for example, under analysis conditions shown in chart 1 of Figure 3.

[0108]

Therefore, the catechin purity can be determined according to Equation ④, and also the recovery rate of tea catechins or caffeine can be calculated.

[0109]

On the other hand, since the catechin oxidative polymerization products and impurities are composed of a plurality of components, the analysis thereof by a usual method generally requires a large amount of work.

[0110]

Actually, in the analysis in the early stage of the development, the present inventors selected mainly saccharides and amino acids as typical components of impurities and measured these components by a usual analytical method for only confirming that these typical components were removed.

[0111]

Furthermore, since it was predicted from knowledge of literatures and so on that the amount of catechin oxidative polymerization products is small, if present, catechin oxidative polymerization products and impurities are collectively treated as a small amount of impurities contained in end products.

[0112]

However, a subsequent study using mass spectrometry and HPLC analysis confirmed using Equation ④ that products prepared by a chromatography column contain a large amount of catechin oxidative polymerization products.

[0113]

Therefore, it was thought that quantitative recognition of catechin oxidative polymerization products and impurities was necessary in order to manufacture tea catechins in an industrial scale under optimum conditions. Accordingly, behavior of catechin oxidative polymerization products and impurities in chromatography column separation has been further investigated.

[0114]

As a result, it was confirmed that impurities applied to the chromatography column separation were not adsorbed to an adsorbent, such as an adsorbent with the main component of styrene divinylbenzene, a vinyl polymer, a methacrylic acid

ester, or dextran, packed in the chromatography column and were eluted by washing with water. Furthermore, it was gravimetrically confirmed that, in component amount ratio of impurities = (component amount in a recovered liquid in an elution process)/(component amount in a tea extract liquid), η_i : $(C_i(\text{out})/C_i(\text{in}))$, $\eta_i \approx 0$ can be achieved by optimizing conditions for washing with water.

[0115]

Figure 4 is a graph showing a change in the weight of impurities when an adsorbent with the main component of styrene divinylbenzene, Sepabeads SP-850 (Mitsubishi Chemical Industries), was packed in a chromatography column, a tea extract liquid was applied to this column, and the column was washed with water, as an example. The impurities were measured at the chromatography outlet.

[0116]

Catechin oxidative polymerization products can be relatively easily analyzed under HPLC analysis conditions that have been newly developed by the present inventors (however, since it is difficult to obtain standard samples of catechin oxidative polymerization products, each component was not quantitatively determined).

[0117]

As an example, HPLC analysis conditions are shown in chart 2 of Figure 5. Figure 6 is a graph showing a breakthrough curve of components that was obtained by packing an adsorbent with the main component of methacrylic acid ester, HP1MG, to a

chromatography column and continuously applying a tea extract liquid to this column.

[0118]

The present inventors have already separated caffeine and tea catechins by optimizing the loading conditions of a tea extract liquid in the adsorption process, the washing conditions in the washing process with water, and the eluant conditions of the elution process. However, as shown in Figure 6, it has been found that tea catechins, caffeine, and catechin oxidative polymerization products exhibited approximately the same adsorption characteristics when the tea extract liquid is continuously applied to the chromatography column in excess of the optimum loading conditions.

[0119]

In addition, it is not shown in the drawing, tea catechins, caffeine, and catechin oxidative polymerization products also exhibited approximately the same elution characteristics when elution procedure was conducted with a 70 vol% acetone aqueous solution or the like after the breakthrough adsorption procedure.

[0120]

Up to that point, the measurement was conducted by mainly using an adsorbent with the main component of styrene divinylbenzene or a methacrylic acid ester that was thought to be economically superior from the viewpoint of handling, physical strength characteristics, and cost of the adsorbent. In particular, since tea catechins and catechin oxidative polymerization products basically have the same or approximate

chemical structures, it has been thought that both had similar chromatography column separation characteristics.

[0121]

On the basis of the above results, the aforementioned Equation ③ showing the purity of tea catechins can be rewritten to Equation ⑤:

$$\eta_t \cdot Ct(in) / (\eta_t \cdot (Ct(in) + Co(in)) + \eta_f \cdot Cf(in)) \times 100 \dots$$

Equation ⑤,

when a test is conducted under the following chromatography column separation conditions:

$\eta_i = 0$, and

$\eta_o = \eta_t$.

[0122]

In Equations ④ and ⑤, each parameter other than $Co(in)$ can be simply determined quantitatively, and Equations ④ and ⑤ are identical within measurement errors. Consequently, the component amount $Co(in)$ of catechin oxidative polymerization products in a tea extract liquid can be calculated. Furthermore, the amount of impurities, $Ci(in)$, in the tea extract liquid can be determined by substituting these values for the aforementioned Equation ①.

[0123]

Therefore, the purity and the like of tea catechins can be analyzed by Equation ⑤ using data obtained under the HPLC conditions shown in chart 1 by previously measuring the composition of tea extract liquid components according to the above-described proceeding. This can significantly reduce the time for the subsequent analysis.

[0124]

The present inventors have conducted further investigation on chromatography column separation and have found a method for removing catechin oxidative polymerization products (Japanese Patent Laid-Open No. 2-311390) and, furthermore, a method for separating caffeine by washing with water (Japanese Patent Application No. 4-113161).

[0125]

In particular, it has been found that the latter method can industrially manufacture tea catechins using an adsorbent with the main component of a vinyl polymer.

[0126]

However, the obtained measurement data was analyzed using Equation ⑤ by the preconception that behavior of tea catechins and catechin oxidative polymerization products are the same when an adsorbent with the main component of a vinyl polymer is used and when an adsorbent with the main component of styrene divinylbenzene or methacrylic acid ester is used.

[0127]

However, in an efficacy evaluation test of the obtained product, excellent data exceeding an error range has been obtained. With such reasons, this time, catechin oxidative polymerization products were also measured under the HPLC conditions shown chart 2 of Figure 5 in order to surely grasp the adsorption and elution characteristics of the adsorbent with the main component of a vinyl polymer.

[0128]

As a result, it has been unexpectedly found that the characteristics of tea catechins and catechin oxidative

polymerization products against the adsorbent with the main component of a vinyl polymer are completely different from each other.

[0129]

Figure 7 shows an example of the above. Figure 7 is a graph showing a breakthrough curve of components obtained by packing an adsorbent of a vinyl polymer, specifically, HW40EC, an adsorbent with the main component of a hydrophilic vinyl polymer, in a chromatography column and continuously applying a tea extract liquid to the column.

[0130]

As shown in the figure, it was confirmed that the adsorption behavior of catechin oxidative polymerization products is similar to that of caffeine rather than that of tea catechins and that the breakthrough of the catechin oxidative polymerization products is ahead of that of tea catechins.

[0131]

As described above, since it is difficult to obtain standard samples of all of catechin oxidative polymerization products, quantitative determination itself is difficult. However, component amount ratio of catechin oxidative polymerization products, (each component amount in a recovered liquid of the elution process)/(each component amount in a tea extract liquid), η_o : $(Co(out)/Co(in))$, can be determined based on a ratio of the integrated absorbance values of the respective peaks that are proportional to the corresponding component concentrations.

[0132]

Therefore, the analysis of the adsorbent with the main component of a vinyl polymer becomes possible by substituting η_0 for the aforementioned Equation ③.

[0133]

Here, each example in Japanese Patent Application No. 4-113161 was reinvestigated by changing the analysis data. The results are shown below together with these Examples.

[0134]

Examples 1, 2, 3, and 4, Comparative Examples 1 and 2, and Reference Example 1 described below correspond to the first aspect of the invention, and Example 5 and Comparative Example 3 correspond to the second aspect of the invention.

[0135]

[Example 1] One kilogram of third tea (samban cha) was extracted with 15 kg of hot water of 95°C for 10 minutes, followed by filtration, concentration under reduced pressure, and centrifugation to prepare a tea extract liquid.

[0136]

A chromatography column was packed with 1 L of Toyopearl HW40 (trade name, Tosoh), which is a commercially available adsorbent with the main component of a hydrophilic vinyl polymer. Then, 3 L of a 12 Brix% tea extract liquid, 5 L of water, and 2.5 L of a 70 vol% ethanol aqueous solution were sequentially applied to the column.

[0137]

The resulting recovered ethanol solution was concentrated under reduced pressure and lyophilized to give 143 g of a solid powder.

[0138]

The results of analysis were that the purity of the target tea catechins was 72 wt% and that of caffeine was 0.9 wt%. The residue was presumed to be catechin oxidative polymerization products. Furthermore, the recovery rate of the tea catechins was 95%.

[0139]

Here, the amounts of tea catechins and caffeine were calculated by dividing the quantitative value determined by an absolute calibration curve of a sample using high-performance liquid chromatography (HPLC) by the amount of the solid powder.

[0140]

Figure 8 shows the chromatogram of HPLC, and chart 3 of Figure 9 shows the HPLC conditions.

[0141]

The recovery rate was calculated by dividing the amount of tea catechins contained in the solid power by the amount of tea catechins contained in the tea extract liquid.

[0142]

By analyzing again according to Equation ③ and others, this Example was presumed that, actually, the purity of the target catechin was about 97.6 wt% and that the contents of caffeine and catechin oxidative polymerization products were about 1.2 wt% and 1.1 wt%, respectively. Furthermore, the product weight estimated based on the analysis data was about 105 g.

[0143]

[Example 2] The method of preparing the tea extract liquid and the adsorbent were the same as those in Example 1.

[0144]

Three liters of an 8 Brix% tea extract liquid, 3 L of water, and 3 L of a 50 vol% methanol aqueous solution were sequentially applied to a chromatography column.

[0145]

As a result, 97 g of a solid powder was obtained. The purity of the tea catechins was 73 wt%, and that of caffeine was 0.2 wt%. The residue was catechin oxidative polymerization products. The recovery rate of the tea catechins was 98%.

[0146]

By analyzing again according to Equation ③ and others, this Example was presumed that, actually, the purity of the target catechin was about 99.5 wt% and that the contents of caffeine and catechin oxidative polymerization products were about 0.3 wt% and 0.2 wt%, respectively. Furthermore, the product weight estimated based on the analysis data was about 71 g.

[0147]

[Comparative Example 1] The method of preparing the tea extract liquid and the adsorbent were the same as those in Example 1.

[0148]

A chromatography column was packed with 1 L of S-876 (trade name) manufactured by Duolite, which is a commercially available adsorbent with the main component of styrene divinylbenzene. Then, 3 L of an 8 Brix% tea extract liquid, 2 L of a 10 vol% methanol aqueous solution, and 3 L of a 50 vol% methanol aqueous solution were sequentially applied to the column.

[0149]

As a result, 94 g of a solid powder was obtained. The purity of tea catechins was 63 wt%, and that of caffeine was 13 wt%. The residue was presumed to be catechin oxidative polymerization products. The recovery rate of the tea catechins was 95%.

[0150]

It was recognized that this separation condition, namely, 2 L of a 10 vol% methanol aqueous solution, was insufficient for desorbing caffeine from the adsorbent. Consequently, only 13% of the total amount of caffeine contained in the tea extract liquid was removed.

[0151]

[Reference Example 1] The tea extract liquid was prepared by the same method as that in Example 1.

[0152]

A chromatography column was packed with 1 L of HP1MG (trade name) manufactured by Mitsubishi Chemical Industries, Ltd., which is a commercially available synthetic adsorbent with the main component of methacrylic acid ester. Then, 3 L of an 8 Brix% tea extract liquid, 6 L of a 10 vol% methanol aqueous solution, and 3 L of a 50 vol% methanol aqueous solution were sequentially applied to the column.

[0153]

As a result, 98 g of a solid powder was obtained. The purity of tea catechins was 71 wt%, and that of caffeine was 1.3 wt%. The residue was presumed to be catechin oxidative polymerization products. The recovery rate of the tea catechins was 97%.

[0154]

In addition, a similar result was obtained by using acetone as a solvent instead of methanol, and the amount of the solvent used could be reduced by 5% of that of methanol.

[0155]

A similar result was obtained when Duolite S-876 (trade name) used in Comparative Example was used as the adsorbent instead of HP1MG (trade name).

[0156]

It was recognized that caffeine was desorbed from the adsorbent with the main component of methacrylic acid ester or styrene divinylbenzene by using this separation condition, namely, 6 L of a 10 vol% methanol aqueous solution.

[0157]

However, the solvent must be collected from 6 L of the 10 vol% methanol aqueous solution, which is a volume three times that in Comparative Example.

[0158]

It was thus confirmed that caffeine can be removed with water at a high efficiency of 95 to 99% by using a gel filtration adsorbent with the main component of hydrophilic polyvinyl alcohol.

[0159]

The behavior of catechin oxidative polymerization products against an adsorbent with the main component of styrene divinylbenzene is the same as that of tea catechins, as newly described in the present invention. Therefore, in Comparative

Example 1 and Reference Example 1 described here, the analytical method hitherto used was recognized being able to be used.

[0160]

It was thus confirmed that caffeine can be removed with water at a high efficiency of 95 to 99% by using a gel filtration adsorbent with the main component of hydrophilic polyvinyl alcohol.

[0161]

The caffeine content in the thus obtained tea catechins is less than 1 wt%. This content was significantly low, namely, 0.01 to 0.1 times that when caffeine is not removed.

[0162]

It was confirmed that an adsorbent with the main component of styrene divinylbenzene or methacrylic acid ester could remove caffeine by optimizing the conditions, as shown in Comparative Example 1. However, it was difficult to remove caffeine only by washing with water as in a case of using an adsorbent of hydrophilic polyvinyl alcohol.

[0163]

[Example 3] One point four kilograms of green tea was extracted with 21 kg of hot water of 80°C for 15 minutes. The extract was subjected to filtration and centrifugation to remove insoluble solids, followed by concentration with RO membrane to give 2.2 L of a 15 Brix% tea extract liquid.

[0164]

Two liters of this tea extract liquid was injected into a chromatography column packed with 1 L of Toyopearl HW40EC (trade name). Then, the column was washed with 3 L of water.

[0165]

Lastly, 4 L of a 60 vol% ethanol aqueous solution was injected into the column, and a liquid was collected.

[0166]

Furthermore, the tea extract liquid of the raw material, the water washing fraction, and the recovered ethanol aqueous solution fraction were analyzed by high-performance liquid chromatography (HPLC).

[0167]

The conditions for the high-performance liquid chromatography analysis are shown in chart 4 of Figure 10. In addition, Figures 11, 12, and 13 show chromatograms of the high-performance liquid chromatography as the analysis results.

[0168]

Furthermore, the amount of each component in the tea extract liquid of the raw material and the recovered ethanol aqueous solution fraction was measured by quantitative high-performance liquid chromatography analysis.

[0169]

As a result of the analysis according to the aforementioned Equation ③ and others, the total recovery rate of (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate catechin was 94.2% and the catechin purity was 97.3 wt%.

[0170]

The contents of caffeine and catechin oxidative polymerization products were 2.0 wt% and 0.7 wt%, respectively.

[0171]

The product weight estimated based on the HPLC measurement result was 58.8 g.

[0172]

In addition, a part of the recovered ethanol aqueous solution fraction was measured for solid content. The solid content was 62 g when converted to the total amount of the product.

[0173]

The catechin purity determined by using this solid content data according to the aforementioned Equation ④ was 92.2 wt%.

[0174]

The product weight was different by 5.2% depending on the measurement method. This difference was thought to be a measurement error and was presumed to be caused by the presence of other minor components such as (+)-catechin, (+)-gallocatechin, and (+)-gallocatechin gallate.

[0175]

[Example 4] Two point two kilograms of green tea was extracted with 35 kg of hot water of 80°C for 15 minutes. The resulting extract was subjected to filtration and centrifugation to remove insoluble solids, followed by concentration with RO membrane to give 4.4 L of a 12 Brix% tea extract liquid.

[0176]

Four liters of this tea extract liquid was injected into a chromatography column packed with 1 L of Asahipak GS-520 (trade name). Then, the column was washed with 3 L of water.

[0177]

Lastly, 3 L of a 70 vol% ethanol aqueous solution was injected into the column to obtain a recovered liquid.

[0178]

The amount of each component of the tea extract liquid of the raw material and the recovered ethanol aqueous solution fraction was measured as in Example 3. The total recovery rate of (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate catechin was 90.5% and the catechin purity was 96.4 wt%.

[0179]

The contents of caffeine and catechin oxidative polymerization products were 2.7 wt% and 1.2 wt%, respectively.

[0180]

The product weight estimated based on the HPLC measurement result was 90.0 g.

[0181]

In addition, a part of the recovered ethanol aqueous solution fraction was measured for solid content. The solid content was 98.4 g when converted to the total amount of the product.

[0182]

The catechin purity determined by using this solid content data according to the aforementioned Equation ④ was 87.8 wt%.

[0183]

[Comparative Example 2] Two liters of a tea extract liquid obtained as in Example 3 was injected into a chromatography column packed with 1 L of HP1MG (manufactured by Mitsubishi

Chemical Industries), which is a methacrylic acid ester adsorbent. Then, the column was washed with 1.7 L of water.

[0184]

Lastly, 4 L of a 60 vol% ethanol aqueous solution was injected to obtain a recovered liquid.

[0185]

The amount of each component of the tea extract liquid of the raw material and the recovered ethanol aqueous solution fraction was measured similarly to Example 3. The total recovery rate of (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate catechin was 81.2% and the catechin purity was 59.2 wt%.

[0186]

The contents of caffeine and catechin oxidative polymerization products were 19.1 wt% and 21.7 wt%, respectively.

[0187]

The product weight estimated based on the HPLC measurement result was 83.3 g.

[0188]

A part of the recovered ethanol aqueous solution fraction was measured for solid content to confirm to be 94.4 g when converted to the total amount of the product.

[0189]

The catechin purity determined by using this solid content data according to the aforementioned Equation ④ was 52.2 wt%.

[0190]

Furthermore, the recovered ethanol aqueous solution fraction was recognized to contain insoluble matter, but this insoluble

matter was thought to be precipitate that was generated because the amount of water used in the washing process was small and thereby a part of impurities was insolubilized by the effect of ethanol.

[0191]

As in each of the above-mentioned Examples, impurities including caffeine and catechin oxidative polymerization products can be removed at a high efficiency to give the target tea catechins only by washing with water by using an adsorbent with the main component of a hydrophilic vinyl polymer.

[0192]

On the other hand, when an adsorbent with the main component of methacrylic acid was used, though a part of impurities, such as saccharides and amino acids, could be removed, it was difficult to separate tea catechins from caffeine and catechin oxidative polymerization products.

[0193]

[Example 5] One kilogram of tea was extracted with 10 L of hot water for 10 minutes and was squeezed. The resulting juice was collected and concentrated under reduced pressure and centrifuged to give 1.5 L of a tea extract liquid (tea extract).

[0194]

A chromatography column was packed with 1.5 L of a synthetic adsorbent HP1MG, which is a commercially available adsorbent with the main component of methacrylic acid ester.

[0195]

All of the tea extract was injected into this column, and then the column was washed with 1.5 L of distilled water and 3.5

L of hot water of 90°C. Furthermore, the remaining components were collected with 3 L of an 80 vol% methanol aqueous solution.
[0196]

The manufactured crude tea catechins had a weight of 112 g, a purity of 85 wt%, and 5 wt% of caffeine.
[0197]

In addition, 92% of tea catechins to the tea extract was collected.
[0198]

[Comparative Example 3] The tea extract and the chromatography column were the same as those in Example 5.
[0199]

All of the tea extract was injected into the column, and washing and collection were conducted using 4.5 L of a 15 vol% methanol aqueous solution and 3 L of an 80 vol% methanol aqueous solution.
[0200]

The manufactured crude tea catechins had a weight of 121 g, a purity of 79 wt%, and 12 wt% of caffeine.
[0201]

In addition, 92% of tea catechins to the tea extract was collected.
[0202]

In this Comparative Example 3, when the washing was conducted using 4.5 L of hot water of 40°C instead of 4.5 L of the 15 vol% methanol aqueous solution, the crude tea catechins had 18 wt% of caffeine. Thus, the washing effect was low.
[0203]

The amount of the crude tea catechins manufactured in the aforementioned Example 5 was 112 g. This was smaller than the amount, 121 g, manufactured in this Comparative Example 3. This was because that caffeine was not contained in the former case. Thus, the present invention was confirmed to be economically effective not to require the collection of low-concentration solvent and also to be excellent in removal of caffeine.

[0204]

[Advantages of the Invention]

According to the present invention as described above, impurities contained in tea leaves are removed with water or hot water and a solvent, such as ethanol, safely from food-hygienic point of view, and tea catechins of high purity can be manufactured at a high recovery rate in an industrial scale.

[0205]

In particular, caffeine and catechin oxidative polymerization products, which are components of which problems are indicated in food-hygienic safety and separation technique depending on removing processes in conventional methods, can be inexpensively removed only by chromatography column separation procedure.

[0206]

In addition, impurities in a tea extract liquid can be removed with only water or hot water. That is, a low-concentration eluant, which is conventionally necessary, is not used. Therefore, facilities for collecting solvents of low-concentration eluants are not necessary, and thereby products can be provided more inexpensively.

[0207]

[Brief Description of the Drawings]

[Figure 1]

Figure 1 is an explanatory diagram schematically illustrating processes of a method for manufacturing tea catechins according to a first aspect of the invention.

[Figure 2]

Figure 2 is an explanatory diagram schematically illustrating processes of a method for manufacturing tea catechins according to a second aspect of the invention.

[Figure 3]

Figure 3 is chart 1 of conditions in high-performance liquid chromatography (HPLC) analysis for quantitatively determining tea catechins.

[Figure 4]

Figure 4 is a graph showing a change in the weight of impurities when an adsorbent with the main component of styrene divinylbenzene, Sepabeads SP-850 (Mitsubishi Chemical Industries), was packed in a chromatography column, a tea extract liquid was applied to this column, and the column was washed with water, and the impurities were measured at the chromatography outlet.

[Figure 5]

Figure 5 is chart 2 of conditions in high-performance liquid chromatography (HPLC) analysis for quantitatively determining catechin oxidative polymerization products. (However, since it is difficult to obtain standard samples of catechin oxidative

polymerization products, quantitative determination itself was not performed.)

[Figure 6]

Figure 6 is a graph showing breakthrough curves of components obtained by continuously applying a tea extract liquid to a chromatography column packed with HP1MG, an adsorbent with the main component of methacrylic acid ester.

[Figure 7]

Figure 7 is a graph showing breakthrough curves of components obtained by continuously applying a tea extract liquid to a chromatography column packed with a vinyl polymer adsorbent, in particular, HP40EC, an adsorbent with the main component of a hydrophilic vinyl polymer, and is a graph particularly involved in the present invention.

[Figure 8]

Figure 8 shows a chromatogram of high-performance liquid chromatography according to Example 1 of the first aspect of the invention.

[Figure 9]

Figure 9 is chart 3 of conditions for high-performance liquid chromatography (HPLC) analysis for the above.

[Figure 10]

Figure 10 is chart 4 of high-performance liquid chromatography conditions according to Example 3 of the first aspect of the invention.

[Figure 11]

Figure 11 shows a chromatogram of high-performance liquid chromatography as an analysis result of the above.

[Figure 12]

Figure 12 shows a chromatogram of high-performance liquid chromatography as an analysis result of the above.

[Figure 13]

Figure 13 shows a chromatogram of high-performance liquid chromatography as an analysis result of the above.

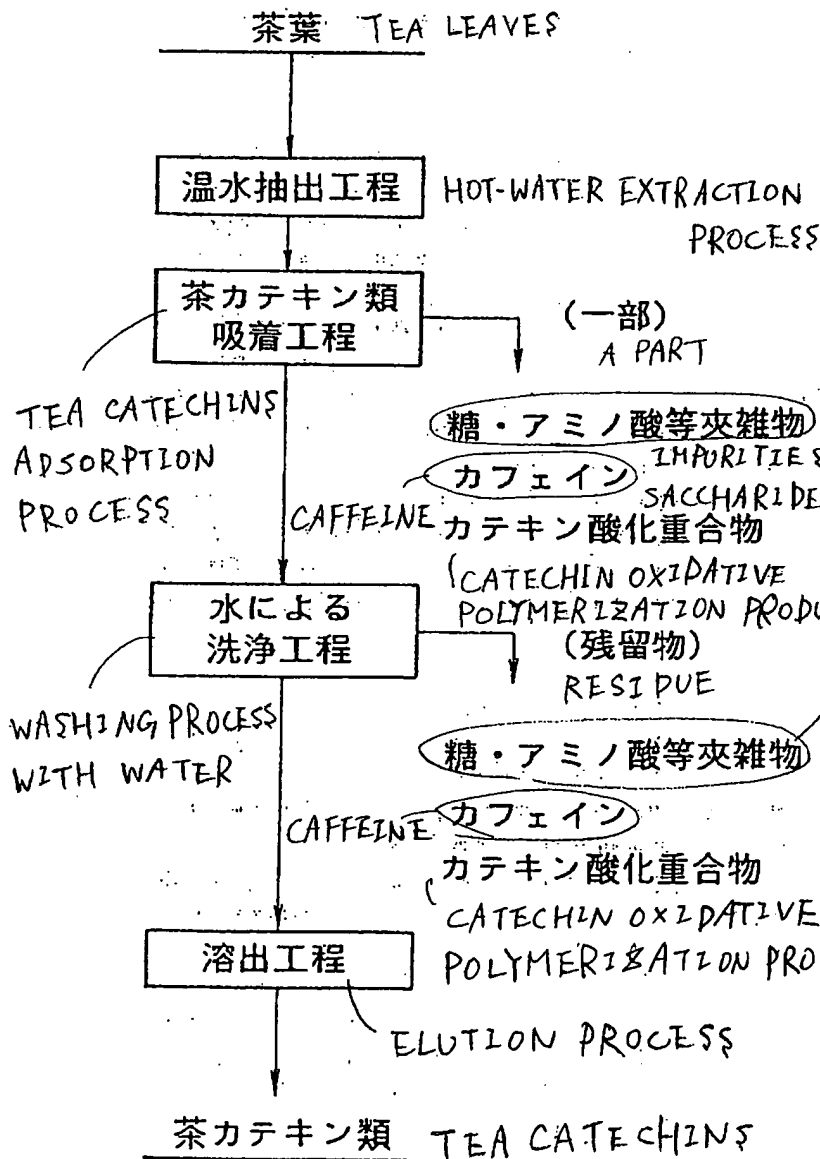
21

速液体クロマトグラフィー (HPLC) の分析条件の図表2である。(ただし、カテキン酸化重合物の標準試料の入手は困難であるためその各成分の定量はしていない)。

【図6】メタアクリル酸エステルを母体とする吸着剤HP1MGをクロマトカラムに充填し、これに茶抽出液を通液し続けることにより得た成分の破過曲線を示すグラフである。

【図7】吸着剤にビニルポリマー、とくに親水性ビニルポリマーを母体とする吸着剤HW40ECをクロマトカラムに充填し、茶抽出液を通液し続けることにより得た成分破過曲線を示す、とくに本発明に関係したグラフである。

【図1】



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【図8】第一の発明の実施例1による高速液体クロマトグラフィーのクロマトグラムを示す図である。

【図9】同、高速液体クロマトグラフィー (HPLC) の分析条件の図表3である。

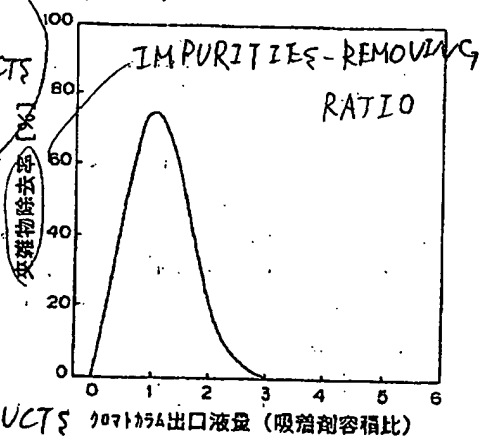
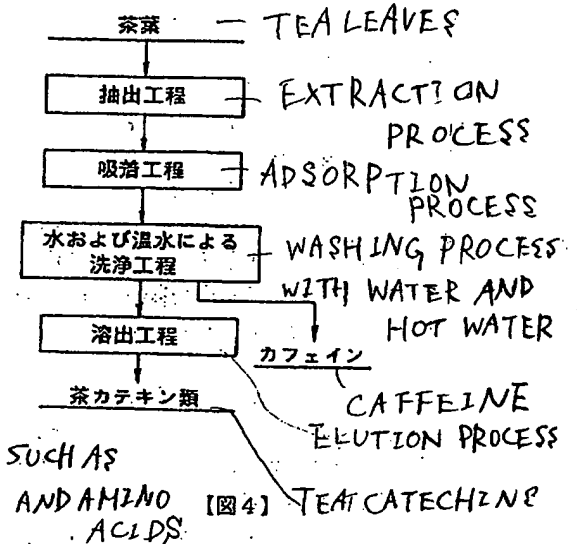
【図10】第一の発明の実施例3における高速液体クロマトグラフィーの分析条件の図表4である。

【図11】同、分析結果として高速液体クロマトグラフィーのクロマトグラムを示す図である。

【図12】同、分析結果として高速液体クロマトグラフィーのクロマトグラムを示す図である。

【図13】同、分析結果として高速液体クロマトグラフィーのクロマトグラムを示す図である。

【図2】



LIQUID VOLUME AT CHROMATOGRAPHY COLUMN OUTLET (VOLUME RATIO TO ADSORBENT)

CHART 1. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)

(図3)

図表1 HPLC (高速液体クロマトグラフィー) の条件

クロマトカラム: CHROMATOGRAPHY COLUMN	カプセルパックC18 (資生堂) CAPCELL PAK C18 SHISEIDO 4.6 mm ϕ \times 250 mm ϕ DIAMETER LENGTH
溶離液: ELUANT	メタノール/水/リン酸 (22/78/0.1) METHANOL/WATER/PHOSPHORIC ACID
流量: FLOW RATE	1 ml/分
試料量: SAMPLE AMOUNT	5 μ l
検出器: DETECTOR	紫外線吸光光度計 ULTRAVIOLET ABSORPTIOMETER 波長 0 \sim 30分 UV 280 nm WAVELENGTH TO min

CHART 2. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)

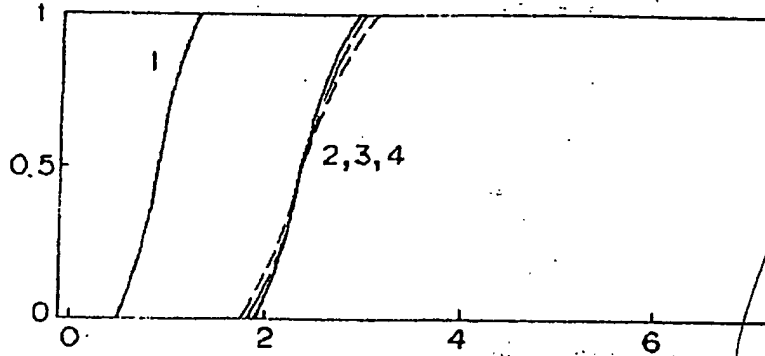
(図5)

図表2 HPLC (高速液体クロマトグラフィー) の条件

クロマトカラム: CHROMATOGRAPHY COLUMN	カプセルパックC18 (資生堂) CAPCELL PAK C18 SHISEIDO 4.6 mm ϕ \times 250 mm ϕ DIAMETER LENGTH
溶離液A: 溶離液B: ELUANT	メタノール/水/リン酸 (22/78/0.1) METHANOL/WATER/PHOSPHORIC ACID
グラジエント: GRADIENT	0 \sim 15分 B液 0% 15 \sim 35分 B液 0 \rightarrow 20% FROM 0% TO 20% 35 \sim 50分 B液 20 \rightarrow 100% FROM 20% TO 100% min
流量: FLOW RATE	1 ml/分 B SOLUTION
試料量: SAMPLE AMOUNT	5 μ l
検出器: DETECTOR	紫外線吸光光度計 ULTRAVIOLET ABSORPTIOMETER 波長 0 \sim 22分 UV 280 nm 22 \sim 50分 UV 350 nm WAVELENGTH TO min

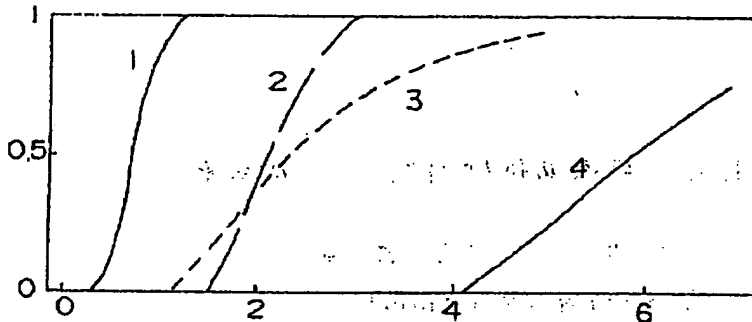
TO

【図6】



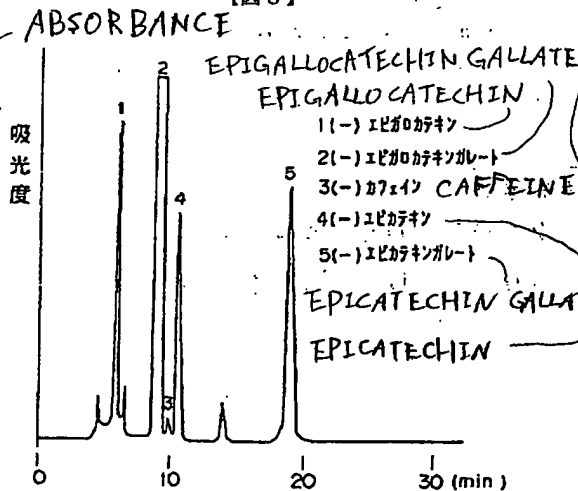
茶抽出液量 (吸着剤容積比)
TEA EXTRACT LIQUID VOLUME
(VOLUME RATIO TO ADSORBENT)

【図7】



茶抽出液量 (吸着剤容積比)
TEA EXTRACT LIQUID VOLUME
(VOLUME RATIO TO ADSORBENT)

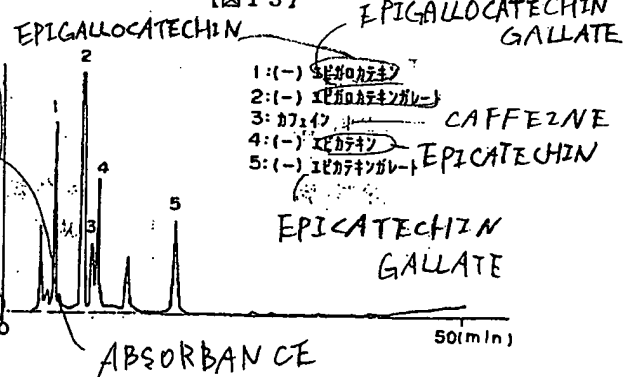
【図8】



EPIGALLOCATECHIN GALLATE
EPIGALLOCATECHIN

1(-) エピガロカテキン
2(-) エピガロカテキンガレート
3(-) カフェイン CAFFEINE
4(-) エピカテキン
5(-) エピカテキンガレート
EPICATECHIN GALLATE
EPICATECHIN

【図13】



EPIGALLOCATECHIN
EPICATECHIN GALLATE
1(-) エピガロカテキン
2(-) エピガロカテキンガレート
3(-) カフェイン CAFFEINE
4(-) エピカテキン
5(-) エピカテキンガレート
EPICATECHIN

CHART 3. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)
 (図9)

図表3 HPLC (高速流体クロマトグラフィー) の条件

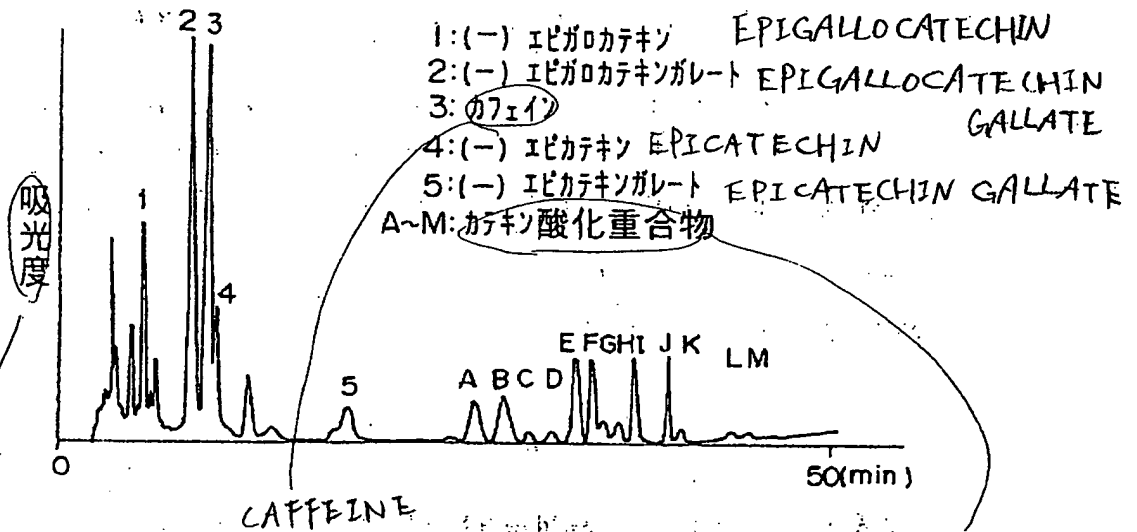
カラム COLUMN	CAPECELLPAK C ₁₈ 4.6 mm φ × 250 mm ¹ 4.6 mm φ × 35 mm ² 40°C
ELUANT 溶離液	Methanol / H ₂ O / H ₃ PO ₄ (22 / 78 / 0.1)
FLOW RATE 流速	1 ml / <u>min</u>
DETECTOR 検出器	UV 280 nm

CHART 4. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)
 (図10)

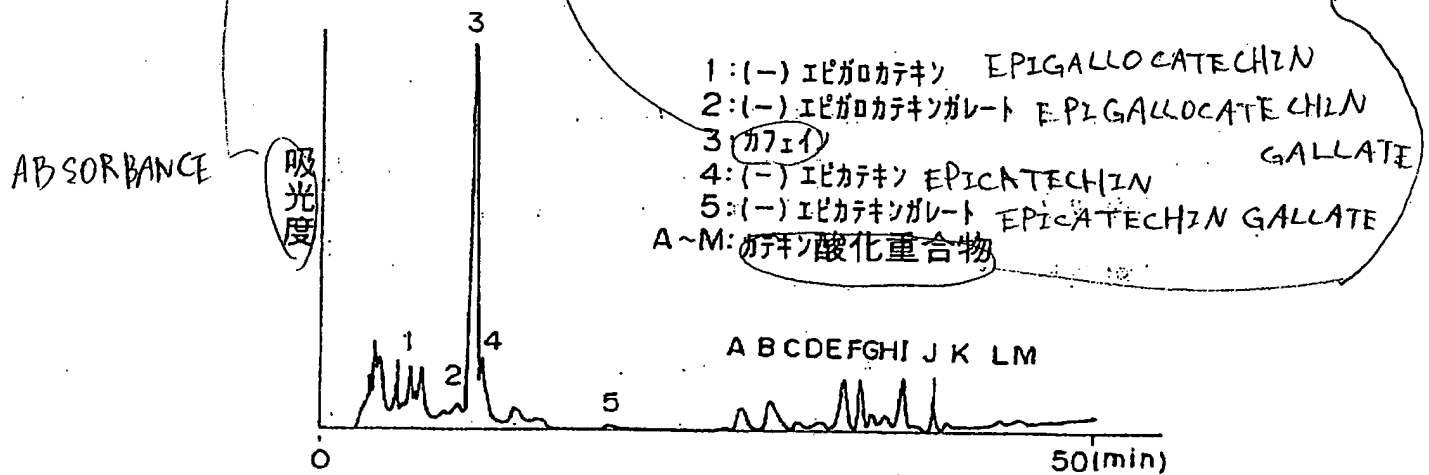
図表4 HPLC (高速流体クロマトグラフィー) の条件

クロマトカラム: CHROMATOGRAPHY COLUMN	CAPCELL PAK C ₁₈ SHISEIDO カプセルバック C18 (資生堂) 4.6 mm <u>径</u> × 250 mm <u>長</u> DIAMETER LENGTH
ELUANT 溶離液A: 溶離液B:	メタノール / 水 / リン酸 (22 / 78 / 0.1) METHANOL / WATER / PHOSPHORIC ACID メタノール METHANOL
GRADIENT グラジエント:	0 ~ 15分 B液 0% 15 ~ 35分 B液 0 → 20% FROM 0% TO 20% 35 ~ 50分 B液 20 → 100% FROM 20% TO 100% TO min B SOLUTION
FLOW RATE 流量:	1 ml / <u>分</u> min
SAMPLE AMOUNT 試料量:	5 μl
DETECTOR 検出器:	ULTRAVIOLET ABSORPTIOMETER 紫外線吸光光度計 波長 0 ~ 22分 UV 280 nm. WAVELENGTH 22 ~ 50分 UV 350 nm TO min

【図11】



【図12】

CATECHIN OXIDATIVE
POLYMERIZATION PRODUCTS

フロントページの続き

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Figure 1

- #1 TEA LEAVES
- #2 HOT-WATER EXTRACTION PROCESS
- #3 TEA CATECHINS ADSORPTION PROCESS
- #4 (A PART)
- #5 IMPURITIES SUCH AS SACCHARIDES AND AMINO ACIDS
- #6 CAFFEINE
- #7 CATECHIN OXIDATIVE POLYMERIZATION PRODUCTS
- #8 WASHING PROCESS WITH WATER
- #9 (RESIDUE)
- #10 ELUTION PROCESS
- #11 TEA CATECHINS

Figure 2

- #1 TEA LEAVES
- #2 EXTRACTION PROCESS
- #3 ADSORPTION PROCESS
- #4 WASHING PROCESS WITH WATER AND HOT WATER
- #5 ELUTION PROCESS
- #6 CAFFEINE
- #7 TEA CATECHINS

Figure 3

- #1 CHART 1. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)
- #2 CHROMATOGRAPHY COLUMN
- #3 CAPCELL PAK C18 (SHISEIDO)
4.6 mm DIAMETER × 250 mm LENGTH

- #4 ELUANT
 - #5 METHANOL/WATER/PHOSPHORIC ACID (22/78/0.1)
 - #6 FLOW RATE
 - #7 1 mL/min
 - #8 SAMPLE AMOUNT
 - #10 DETECTOR
 - #11 ULTRAVIOLET ABSORPTIOMETER
- WAVELENGTH: 0 TO 30 min, UV: 280 nm

Figure 4

- #1 IMPURITIES-REMOVING RATIO [%]
- #2 LIQUID VOLUME AT CHROMATOGRAPHY COLUMN OUTLET (VOLUME RATIO TO ADSORBENT)

Figure 5

- #1 CHART 2. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)
- #2 CHROMATOGRAPHY COLUMN
- #3 CAPCELL PAK C18 (SHISEIDO)
4.6 mm DIAMETER × 250 mm LENGTH
- #4 ELUANT A
- #5 METHANOL/WATER/PHOSPHORIC ACID (22/78/0.1)
- #6 ELUANT B
- #7 METHANOL
- #8 GRADIENT
- #9 0 TO 15 min, B SOLUTION: 0%
15 TO 35 min, B SOLUTION: FROM 0% TO 20%
35 TO 50 min, B SOLUTION: FROM 20% TO 100%

#10 FLOW RATE
#11 1 mL/min
#12 SAMPLE AMOUNT
#14 DETECTOR
#15 ULTRAVIOLET ABSORPTIOMETER
WAVELENGTH: 0 TO 20 min, UV: 280 nm
22 TO 50 min, UV: 350 nm

Figure 6

#1 TEA EXTRACT LIQUID VOLUME (VOLUME RATIO TO ADSORBENT)
#2 1: IMPURITIES
#3 2: CATECHIN OXIDATIVE POLYMERIZATION PRODUCTS
#4 3: CAFFEINE
#5 4: CATECHIN
#6 Cin: COMPONENT AMOUNT AT COLUMN INLET
#7 Cout: COMPONENT AMOUNT AT COLUMN OUTLET

Figure 7

#1 TEA EXTRACT LIQUID VOLUME (VOLUME RATIO TO ADSORBENT)
#2 1: IMPURITIES
#3 2: CATECHIN OXIDATIVE POLYMERIZATION PRODUCTS
#4 3: CAFFEINE
#5 4: CATECHIN
#6 Cin: COMPONENT AMOUNT AT COLUMN INLET
#7 Cout: COMPONENT AMOUNT AT COLUMN OUTLET

Figure 8

#1 ABSORBANCE

- #2 1: (-)-EPIGALLOCATECHIN
- #3 2: (-)-EPIGALLOCATECHIN GALLATE
- #4 3: (-)-CAFFEINE
- #5 4: (-)-EPICATECHIN
- #6 5: (-)-EPICATECHIN GALLATE

Figure 13

- #1 ABSORBANCE
- #2 1: (-)-EPIGALLOCATECHIN
- #3 2: (-)-EPIGALLOCATECHIN GALLATE
- #4 3: CAFFEINE
- #5 4: (-)-EPICATECHIN
- #6 5: (-)-EPICATECHIN GALLATE

Figure 9

- #1 CHART 3. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)
- #2 COLUMN
- #3 ELUANT
- #4 FLOW RATE
- #5 DETECTOR

Figure 10

- #1 CHART 4. CONDITIONS OF HPLC (HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY)
- #2 CHROMATOGRAPHY COLUMN
- #3 CAPCELL PAK C18 (SHISEIDO)
4.6 mm DIAMETER × 250 mm LENGTH

#4 ELUANT A
#5 METHANOL/WATER/PHOSPHORIC ACID (22/78/0.1)
#6 ELUANT B
#7 METHANOL
#8 GRADIENT
#9 0 TO 15 min, B SOLUTION: 0%
15 TO 35 min, B SOLUTION: FROM 0% TO 20%
35 TO 50 min, B SOLUTION: FROM 20% TO 100%
#10 FLOW RATE
#11 1 mL/min
#12 SAMPLE AMOUNT
#14 DETECTOR
#15 ULTRAVIOLET ABSORPTIOMETER
WAVELENGTH: 0 TO 20 min, UV: 280 NM
22 TO 50 min, UV: 350 NM

Figure 11

#1 ABSORBANCE
#2 1: (-)-EPIGALLOCATECHIN
#3 2: (-)-EPIGALLOCATECHIN GALLATE
#4 3: CAFFEINE
#5 4: (-)-EPICATECHIN
#6 5: (-)-EPICATECHIN GALLATE
#7 A TO M: CATECHIN OXIDATIVE POLYMERIZATION PRODUCTS

Figure 12

#1 ABSORBANCE
#2 1: (-)-EPIGALLOCATECHIN

- #3 2: (-)-EPIGALLOCATECHIN GALLATE
- #4 3: CAFFEINE
- #5 4: (-)-EPICATECHIN
- #6 5: (-)-EPICATECHIN GALLATE
- #7 A TO M: CATECHIN OXIDATIVE POLYMERIZATION PRODUCTS